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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>5</sup> :</b> <b>C12Q 1/48, G01N 33/573</b> <b>C07K 15/00 // C12P 21/08</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 93/22452</b> <b>(43) International Publication Date:</b> 11 November 1993 (11.11.93)
<b>(21) International Application Number:</b> PCT/IE93/00018 <b>(22) International Filing Date:</b> 31 March 1993 (31.03.93)  <b>(30) Priority data:</b> 921354 1 May 1992 (01.05.92) <b>IE</b>  <b>(71)(72) Applicants and Inventors:</b> KILTY, Cormac, Gerard [IE/IE]; 31 Monkstown Avenue, Monkstown, County Dublin (IE). O'BYRNE, Seamus [IE/IE]; 16 The Alders, Monkstown Valley, Monkstown, County Dublin (IE).  <b>(74) Agent:</b> ANNE RYAN & CO.; 60 Northumberland Road, Ballsbridge, Dublin 4 (IE).		<b>(81) Designated States:</b> JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> MEASUREMENT OF AN ENZYME MARKER AS AN AID TO DIAGNOSIS  <b>(57) Abstract</b>  A method which assists in the early diagnosis of rejection in a liver transplant recipient comprises measuring an increase in plasma or serum <i>alpha</i> glutathione S-transferase ( $\alpha$ -GST) from said recipient in the absence of or preceding any change in plasma or serum transaminase. $\alpha$ -GST is most suitably measured by enzymeimmunoassay, using a solid phase antibody which is monospecific for $\alpha$ -GST. The monospecific antibody cross-reacts with the $\alpha$ -GST dimers B <sub>1</sub> B <sub>1</sub> , B <sub>1</sub> B <sub>2</sub> and B <sub>2</sub> B <sub>2</sub> .		

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## Description

### Measurement of an enzyme marker as an aid to diagnosis

#### Technical Field

5 This invention relates to a method which assists in the early diagnosis of rejection in a liver transplant recipient.

#### Background Art

10 In liver transplant recipients the risk of allograft rejection is greatest in the first few weeks after transplantation, although it can occur as late as the 8th post-operative month. Rejection is most common, however, between the 4th and 10th post-operative days. Prompt diagnosis is crucial to limit damage by this allogeneic immune response. It is also vital that the diagnosis is correct, since administration of augmented immunosuppression in the absence of rejection has its own morbidity - particularly in delaying wound  
15 healing and in predisposing the patient to serious infection. The most reliable evidence of rejection is histological but may not always be possible due to severe impairment of clotting. In current practice, the suspected diagnosis of rejection usually rests on evidence of progressive deterioration of liver function in the absence of any other explanation  
20 for this functional derangement (Calne, Ry, (1987); Liver Transplantation (2nd Edition), Ed Calne Ry, Grune & Stratton, Inc., London, 301-303). Thus, significant rejection is not diagnosed unless the serum bilirubin and alkaline phosphatase levels are elevated. If the serum transaminases and prothrombin time are also rising, then  
25 rejection is assumed unless there is evidence of (a) portal vein/hepatic artery obstruction, (b) septicaemia or (c) drug toxicity. The duration of treatment of rejection by augmented immunosuppression will depend on the improvement in liver function tests (LFTs). Patients with persistently raised LFTs have a poor prognosis.

The biochemical assessment of liver function usually includes measurement of plasma or serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT) activity. These cytosolic enzymes are released into the circulation following hepatocellular damage. The measurement of these aminotransferases for monitoring liver function has been questioned, however, as activities may be normal in patients with chronic liver disease. The poor sensitivity of aminotransferases in detecting damage in certain types of liver pathology may partly lie in their distribution within the liver. The periportal hepatocytes contain the highest concentrations of the aminotransferases but the centrilobular hepatocytes, which are relatively deficient in aminotransferases, are more susceptible to damage from hypoxia and toxins such as alcohol and paracetamol.

Recently, the measurement of hepatic alpha glutathione S-transferase ( $\alpha$ -GST) has been advocated as a superior marker of hepatocellular damage than the aminotransferases in a variety of clinical conditions including halothane hepatotoxicity (Hussey, A.J. *et al.* (1988); Br. J. Anaesth., 60, 130-135), autoimmune chronic active hepatitis (Hayes, P.C. *et al.* (1988); Clin. Chim. Acta., 172, 211-216), birth asphyxia (Beckett, G.J. *et al.* (1989) Clin. Chem. 35, 995-999) and paracetamol poisoning (Beckett, G.J. *et al.* (1989); Clin. Chem., 35, 2186-2189). Indeed, studies of acute liver damage and chronic active hepatitis have indicated that GST activities, unlike those of transaminase, correlate better with histological abnormalities. (Bass, N.M. *et al.* (1978); Gastroenterology, 75, 589-594 and Sherman, M. *et al.* (1983); Hepatology, 3, 162-169).

The GSTs are a complex family of enzymes involved in detoxification. The enzymes catalyze the nucleophilic attack of glutathione (GSH) on a wide range of hydrophobic electrophiles. The GSTs can be divided into three classes: the basic, the near-neutral and the acidic enzymes according to their isoelectric points. These classes are related to the alpha (I), mu (II) and pi (III) families, respectively, in the rat (2). The human alpha class enzymes, also referred to as

"ligandin" (3), comprise two subunits B<sub>1</sub> and B<sub>2</sub> which can hybridize to form the homodimers, B<sub>1</sub>B<sub>1</sub> and B<sub>2</sub>B<sub>2</sub> and the heterodimer, B<sub>1</sub>B<sub>2</sub> (4).

Adult liver contains predominantly the basic or  $\alpha$ -GST. One of the properties of hepatic GST which may partly explain its greater sensitivity as a marker of liver damage when compared with the aminotransferases is its wider distribution within the liver. Immunohistochemical studies of GST in human fetuses, neonates and adults have shown that the basic and acidic GST are equally expressed in both periportal and centrilobular hepatocytes (Hiley C. *et al.* (1988); Biochem. J., 284, 255-259). Other properties of GSTs also offer theoretical advantages over measurements of aminotransferases in the investigation of liver damage. They are relatively small enzymes (MW ~ 50,000) and are present in high concentrations in the hepatocyte cytosol. GSTs are readily and rapidly released in quantity into the circulation following hepatic damage; their short plasma half-life (<90 min.) allowing early detection of hepatic damage and its resolution.

Histologically the acute rejection process appears to begin with a mononuclear cell infiltration of portal tracts. It is noteworthy that spillover of lymphocytes into the adjacent periportal parenchyma is occasionally but not always a feature of acute rejection. There may also be involvement of centrilobular blood vessels and bile ducts. The pattern of infiltration within the liver is also variable - ranging from focal to diffuse. The diagnostic sensitivity of the transaminases as early markers of rejection depends on their distribution within the liver in relation to those sites involved at the beginning of the rejection process. If the periportal hepatocytes are not affected in this early pathological process, changes in transaminases may not always be expected to rise markedly and diagnosis and treatment could be delayed. This may be particularly important in the first few days after transplantation, when the patient is at greatest risk of rejection and transaminases remain elevated as a result of ischaemia and surgical trauma to the liver.

It is an object of the present invention to provide a method which assists in the early diagnosis of rejection in liver transplant recipients.

It is a further object of the present invention to provide a more sensitive and specific marker of the rejection process in the liver transplant recipient than has heretofore been available.

5 It is a still further object of the present invention to provide a method for the monitoring of post-operative liver transplant patients so that earlier corrective action can be taken which may prevent rejection occurring.

#### Disclosure of Invention

10 Accordingly, the invention provides a method which assists in the early diagnosis of rejection in a liver transplant recipient, which method comprises measuring an increase in plasma or serum *alpha* glutathione S-transferase ( $\alpha$ -GST) from said recipient in the absence of or preceding any change in plasma or serum transaminase.

Preferably, the  $\alpha$ -GST is measured by enzymeimmunoassay.

15 In a particularly preferred embodiment of the invention there is used as solid phase an antibody which is monospecific for  $\alpha$ -GST, more especially a monospecific antibody which cross-reacts with the  $\alpha$ -GST dimers B<sub>1</sub>B<sub>1</sub>, B<sub>1</sub>B<sub>2</sub> and B<sub>2</sub>B<sub>2</sub>.

20 Further, preferably, the antibody for use in accordance with the invention does not cross-react with either GST-mu or GST-pi.

The enzymeimmunoassay for use in accordance with the invention is preferably one that can quantify the level of  $\alpha$ -GST in human plasma or serum to a sensitivity limit of 0.75ng/ml.

25 Also in accordance with the invention, the enzymeimmunoassay enables one to measure an increase in  $\alpha$ -GST within a time period of less than five hours, more especially less than four hours.

The invention also provides a polyclonal antibody for use in a method according to the invention as hereinbefore defined and which cross-reacts with the  $\alpha$ -GST dimers B<sub>1</sub>B<sub>1</sub>, B<sub>1</sub>B<sub>2</sub> and B<sub>2</sub>B<sub>2</sub>.

5 It will be appreciated that a monoclonal antibody having the requisite specificity can also be used in the method according to the invention.

Such a monoclonal antibody may suitably be human, mouse or rat monoclonal antibody prepared by conventional methods, including those methods currently available for producing monoclonal antibody on a commercial scale, genetically engineered monoclonal antibody or antibody fragments or antibody produced by *in vitro* immunisation of suitable cells.

15 An enzymeimmunoassay in accordance with the invention can be carried out using any known format, such as, for example beads, dip-sticks, membranes, particles, plates, rods, strips, tubes or wells.

For example, insolubilised or solid phase antibody as used herein is suitably bound to a bead, dip-stick, membrane, particle, plate, rod, strip, tube or well of plastics material or glass in a manner known *per se*. More specifically, the insolubilised form of the antibody comprises said antibody adsorbed on a surface adapted for protein adsorption such as a bead, dip-stick, membrane, particle, plate, rod, strip, tube or well.

In an especially preferred embodiment, the surface comprises a plastics microtitration plate or strip adapted for protein adsorption wherein the immunochemical reaction and the estimation of  $\alpha$ -GST can take place. Especially suitable microtitration plates are gamma-irradiated microtitration plates. Examples of such gamma-irradiated microtitration plates are flat-well polystyrene microtitration plates marketed by DYNATECH under the Trade Mark MICROELISA and those sold under the Trade Mark "NUNC" IMMULON. Examples of strips are strips marketed by DYNATECH under the Trade Mark REMOVAWELL.

The relevant surface may be coated directly with an optimum dilution of polyclonal antibody prepared by separating the relevant immunoglobulin fraction of antiserum.

5 The estimation of the bound  $\alpha$ -GST is preferably carried out by enzymeimmunoassay using an enzyme-label. The labelled enzyme can be prepared in conventional manner or purchased from appropriate suppliers. A suitable enzyme label is an enzyme conjugate comprising an enzyme-labelled antibody for use in a competitive binding assay.

10 The estimation of the bound  $\alpha$ -GST is preferably carried out by enzymeimmunoassay using a suitable peroxidase labelled antibody or other suitable peroxidase conjugate. A suitable peroxidase is horse radish peroxidase. One such other suitable peroxidase conjugate is an avidin-biotin peroxidase complex, which can be used with an antibody biotin conjugate to amplify the enzyme assay in conventional manner.

15 In such an enzyme assay antigen insolubilised on solid phase antibody binds to the antibody-biotin complex which in turn binds to the avidin/streptavidin-biotin peroxidase complex, whereupon the peroxidase activity is measured.

20 The estimation of the bound  $\alpha$ -GST can also be carried out by fluorometric, luminometric or radiometric assay, using fluorochromes, light-emitting probes or radio labels, respectively.

#### Brief Description of Drawings

25 Fig. 1 is a graph of multiple upper limit of normal range for a number of markers, including  $\alpha$ -GST, *versus* day post liver transplant for a first recipient;

Fig. 2 is a graph of multiple upper limit of normal range for a number of markers, including  $\alpha$ -GST, *versus* day post liver transplant for a second recipient;



Fig. 3 is a graph of multiple upper limit of normal range for a number of markers, including  $\alpha$ -GST, *versus* day post liver transplant for a third recipient; and

5 Fig. 4 is a graph of multiple upper limit of normal range for a number of markers, including  $\alpha$ -GST, *versus* day post liver transplant for a fourth recipient;

In each of Figs. 1-4,  $\square-\square$  represents bilirubin (BILI),  $\bullet-\bullet$  represents alkaline phosphatase (ALP),  $\circ-\circ$  represents ALT,  $\diamond-\diamond$  represents AST and  $\blacksquare-\blacksquare$  represents  $\alpha$ -GST.

10 The invention will be further illustrated by the following Examples.

#### Best Modes for Carrying Out the Invention

##### EXAMPLE 1

Polyclonal antibody production.

##### 15 1) Antigen purification

Approximately 1g of human liver was homogenised in 3 volumes of a homogenising buffer at pH 7.2 having the following composition:

20 0.25M sucrose  
10mM Tris/HCl  
1mM EDTA

in a Waring Blender for 3 min. The homogenate was centrifuged at 16,000g for 20 min. and the supernatant decanted and centrifuged at 45,000g for 1h. The resulting supernatant was decanted and the

retentate filtered through glass wool, followed by dialysis against 20 litres 10mM Tris, pH 7.8, 0.2M NaCl (buffer 1) overnight at 4°C. The dialysate was centrifuged at 16,000g for 10 min. The supernatant was then applied to an S-hexylGSH-agarose column equilibrated in buffer 1 and washed with buffer 1 until  $E_{280}=0$ .

The column was eluted with 0.03mM S-hexylGSH in buffer 1.

The protein peaks ( $\alpha$ -GST-basics pool) were pooled and the pooled material dialysed against 2 x 5 litres 10mM sodium phosphate buffer at pH 7.0. The dialysate was checked by SDS PAGE, isoelectric focussing (urea) and enzyme activity in hydrolysing chloro-2,4-dinitrobenzene (cDNB).

Yield  $\approx$  30mg protein.

The  $\alpha$ -GST was stored in aliquots at -20°C until required for use.

## 2) Antisera production

Day 1.

200 $\mu$ g of  $\alpha$ -GST obtained in step 1 in 0.5ml buffer 1 and 0.5ml Freund's complete adjuvant was vortexed for 2h. to form an emulsion. The antigen emulsion was infection intradermally into multiple sites on to the previously shaved back of a rabbit.

Day 21.

The procedure at day 1 was repeated except that the antigen emulsion was injected intramuscularly into the leg of the rabbit.

Day 31.

The procedure for day 21 was repeated.

Day 38.

Blood samples were collected from an ear vein. The serum collected and IgG polysera purified on protein A-agarose.

A stock anti- $\alpha$ -GST IgG was prepared containing 2.98 mg/ml.

5

## EXAMPLE 2

### Enzymeimmunoassay.

10

Plasma samples are dispensed (200 $\mu$ l) into duplicate microtitre wells coated with purified monospecific antibody to  $\alpha$ -GST (1 $\mu$ g/well in 0.1M carbonate/bicarbonate buffer, pH 9.6) as prepared in Example 1 and into a number of positive control wells containing a preparation of purified  $\alpha$ -GST from human liver (50ng/ml). The positive controls are diluted with phosphate buffered saline containing TWEEN 20 (25%) (TWEEN is a Trade Mark) (PBST). The positive control panel for the standard curve is prepared as follows:

Final concentration (ng/ml):	25	10	5	2.5	0.75	0
Positive Control ( $\mu$ l):	250	100	50	25	7.5	0
PBST ( $\mu$ l)	250	400	450	475	492.5	500

15

High level plasma samples can be diluted with PBST to bring them onto a linear scale. All wells are then incubated at room temperature for 1 hour.

20

The sample is then removed and the wells are washed with PBST four times.

A working dilution of biotinylated anti-GST is prepared by diluting 1/400 concentrated biotinylated anti  $\alpha$ -GST (1.7mg/ml) with PBST. 50 $\mu$ l of the latter is dispensed into each well and the wells are incubated at room temperature for 1 hour.

5           The solution is removed and the wells are washed with PBST four times.

10           A working dilution of streptavidin biotinylated peroxidase complex is prepared to give a concentration of 0.4 $\mu$ g/ml with peroxidase activity of 0.08 units/ml and 50 $\mu$ l of the latter is dispensed into each well. The wells are again incubated at room temperature for one hour. The latter solution is removed and the wells are washed six times with PBST. Tetramethyl benzidine (TMB) chromogen containing 0.03% hydrogen peroxide in 0.1M citrate/carbonate buffer, pH 5.0 is added to each well (200 $\mu$ l). The colour is allowed to develop  
15           for 15 min. and then the reaction is stopped by adding 2NH<sub>2</sub>SO<sub>4</sub> (50 $\mu$ l). The wells are read at 450nm. The mean results are calculated, the standard curve is graphed and unknown sample values obtained by extrapolation in conventional manner.

An *o*-phenylene diamine (OPD) chromogen can also be used.

20

### EXAMPLE 3

#### Preliminary retrospective study.

25           A retrospective, longitudinal study of liver function in 11 consecutive adult liver transplant recipients, in the period up to 3 months after transplantation has been carried out on our behalf at Addenbrook's Hospital, Cambridge, U.K. by Dr. Andrew Trull. All samples sent for routine LFTs, including serum bilirubin (BILI), alkaline phosphatase (ALP) and ALT were also assayed for serum AST and  $\alpha$ -GST. Longitudinal results were plotted as a multiple of the upper limit of the normal range (ULN) for each analyte to bring  
30           covariate data onto a comparable scale. Each patient's clinical course

was carefully reviewed with particular attention being paid to the documentation of events that might influence liver function. These included rejection, vascular/biliary obstruction, infection, surgical/invasive procedures such as laparotomy or biopsy and the demonstration of potentially hepatotoxic drugs. The diagnosis of rejection was usually based upon histopathology but in those cases where a biopsy was not carried out, despite clinical evidence of rejection, treatment with augmented immunosuppression was considered diagnostic. In this study the ULN values for the markers measured were as follows:

	BILI	17 $\mu$ moles/litre
	ALP	135 units/litre
	ALT	40 units/litre
	AST	37 units/litre
15	$\alpha$ -GST	10 $\mu$ g/litre

Longitudinal LFT results from four of the liver transplant recipients studied are shown in Figs. 1-4.

Case 1 (Fig. 1) illustrated the importance of the short half-life of  $\alpha$ -GST. High levels of GSTs following transplantation fell rapidly from nearly 35 times the ULN to only 3 times the ULN by the high risk period at day 4. A biopsy taken on day 4 showed no evidence of rejection and  $\alpha$ -GST concentrations continued to fall, consistent with this finding. On day 4 the ALT activity was still 10 times the ULN falling only slowly after transplantation. Good baseline levels of all LFTs were seen until day 15 when Grade 1 rejection was diagnosed histologically. The 3 to 5-fold increases in transaminases at this time compare with a 30-fold increase in  $\alpha$ -GST, illustrating the greater sensitivity of serum  $\alpha$ -GST measurements in the rejection process.

Case 2 (Fig. 2) further illustrates the importance of the short half-life of  $\alpha$ -GST in the early post-operative period. The first rejection episode in this patient, diagnosed by biopsy on day 9, was

5 marked by a 6-fold increase in  $\alpha$ -GST but no comparable rise in other LFTs was found. The changes in  $\alpha$ -GST seen preceding the second biopsy-proven rejection episode on day 17 are also important because marked changes in  $\alpha$ -GSTs were apparent at least 2 days earlier than changes in transaminases.

10 Case 3 (Fig. 3) illustrates the dramatic changes in  $\alpha$ -GST seen in some patients in the absence or preceding any change in transaminases during rejection. Here the peak in  $\alpha$ -GST preceded the ALT peak by 4 days, yet  $\alpha$ -GST levels had returned to normal more than 3 days before the ALT.

Case 4 (Fig. 4) also shows the earlier greater and more defined changes in  $\alpha$ -GST in a patient treated for rejection (without biopsy confirmation) on day 9.

\* \* \* \* \*

15 Although a full clinical review of all 11 cases is still underway overall GST appears to be a more sensitive and specific marker of the early rejection process. Its potential clinical value, predicted from its physicochemical properties, has been substantiated by this longitudinal, retrospective study.  $\alpha$ -GST, as an adjunct to clinical indices of rejection, may prove to be sufficiently specific for rejection to avoid  
20 the requirement for biopsy which has its own morbidity and mortality. It is postulated that the method according to the invention has the potential to improve the management of transplant patients with possible reduction in hospital time. It may also improve the assessment,  
25 of the various medication regimes that a patient must receive particularly allowing the physician to cease administration of potentially damaging treatments at an earlier stage.

\* \* \* \* \*

Thus the expected clinical advantages of the method according to the invention for measurement of hepatic  $\alpha$ -GST in liver transplant recipients over conventional LFTS can be summarised as follows:

- 5           (a) the short half-life of  $\alpha$ -GST may be expected to result in a more rapid fall to normal baseline concentrations following surgically uncomplicated transplantation; potentially improving the resolution of subsequent changes in liver function;
- 10          (b) the development of different patterns of rejection are more likely to be revealed early and more consistently by the relatively large changes in  $\alpha$ -GST due to the enzyme's high cytosolic concentration in hepatocytes and more general distribution throughout the liver; and
- 15          (c) it should be possible to monitor the resolution of rejection following treatment more closely - again due to the short half-life of  $\alpha$ -GST in the circulation. Thus, it may be possible to stop administration of augmented immunosuppression earlier.

20           The invention is not limited to the embodiments described above, which may be modified and/or varied without departing from the scope of the invention.

CLAIMS:

1. A method which assists in the early diagnosis of rejection in a liver transplant recipient, which method comprises measuring an increase in plasma or serum *alpha* glutathione S-transferase ( $\alpha$ -GST) from said recipient in the absence of or preceding any change in plasma or serum transaminase.
2. A method according to Claim 1, wherein the  $\alpha$ -GST is measured by enzymeimmunoassay.
3. A method according to Claim 2, wherein there is used as solid phase an antibody which is monospecific for  $\alpha$ -GST.
4. A method according to Claim 3, wherein the monospecific antibody cross-reacts with the  $\alpha$ -GST dimers  $B_1B_1$ ,  $B_1B_2$  and  $B_2B_2$ .
5. A method according to Claim 3 or 4, wherein the antibody does not cross-react with either GST-mu or GST-pi.
6. A method according to any one of Claims 2-5, wherein the enzymeimmunoassay can quantify the level of  $\alpha$ -GST in human plasma or serum to a sensitivity limit of 0.75ng/ml.
7. A method according to any one of Claims 1-6, wherein the increase in  $\alpha$ -GST can be measured within a time period of less than five hours.
8. A polyclonal antibody for use in a method according to any one of Claims 1-7, and which cross-reacts with the  $\alpha$ -GST dimers  $B_1B_1$ ,  $B_1B_2$  and  $B_2B_2$ .



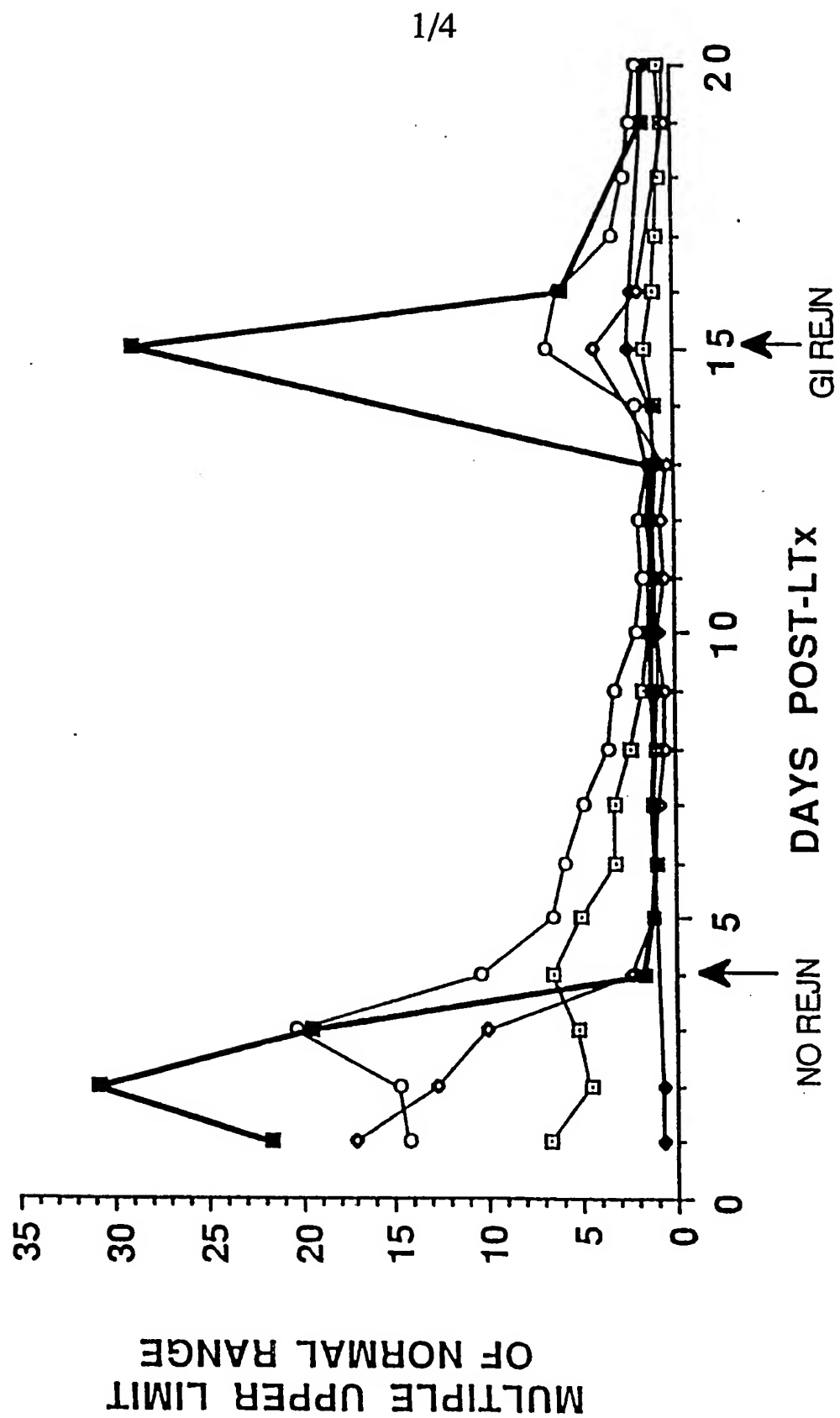


Fig. 1

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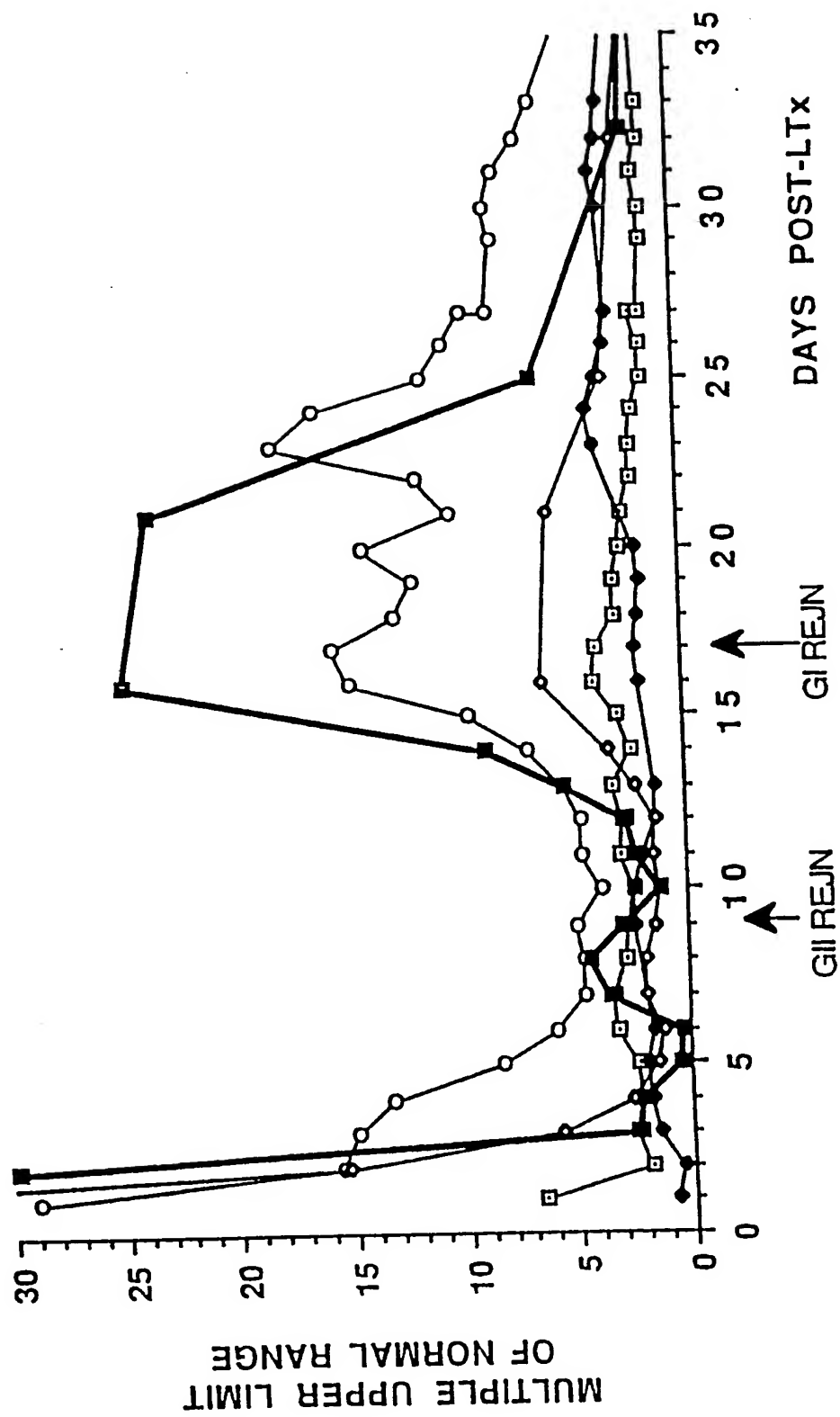


Fig. 2

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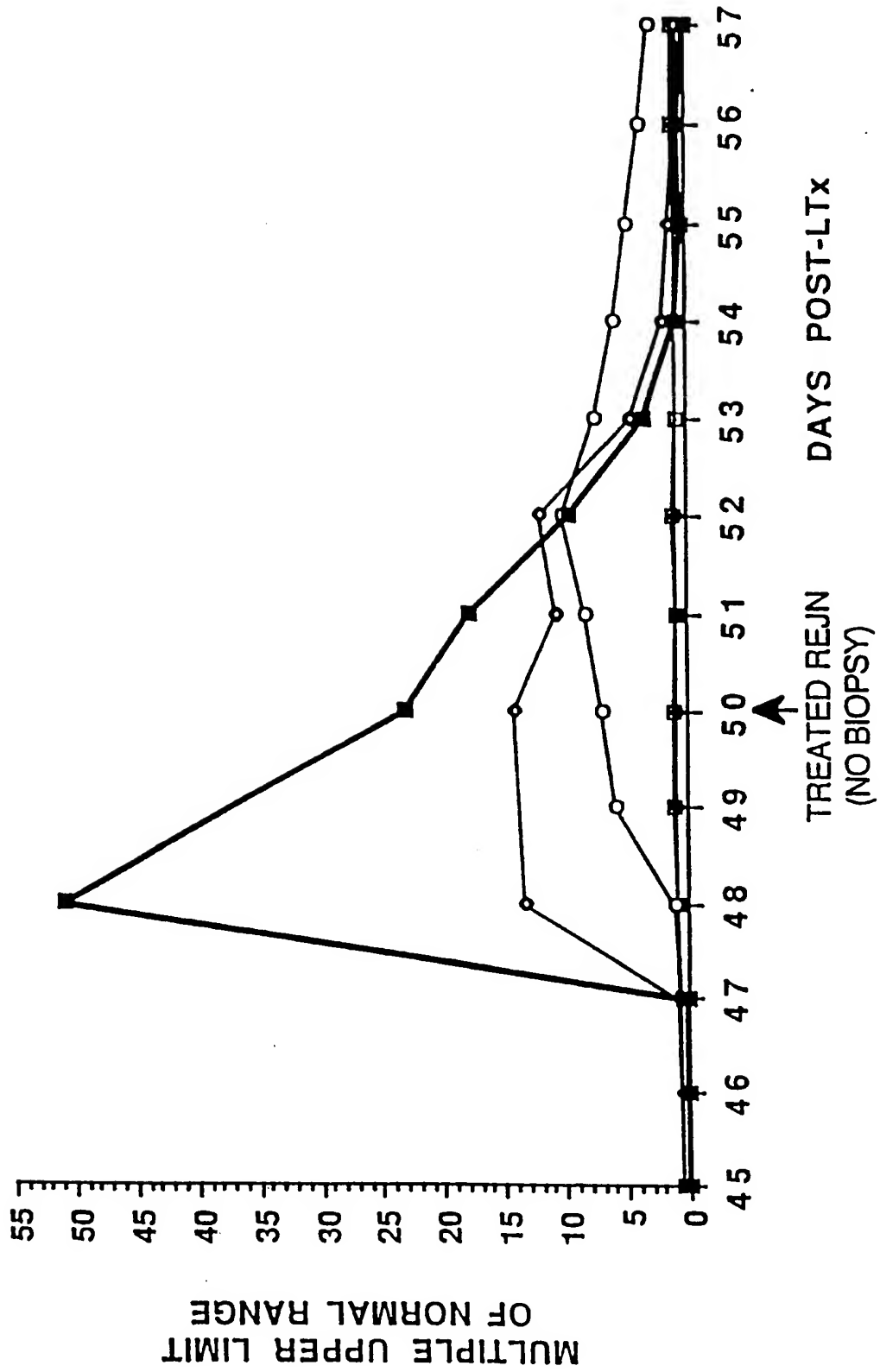


Fig. 3

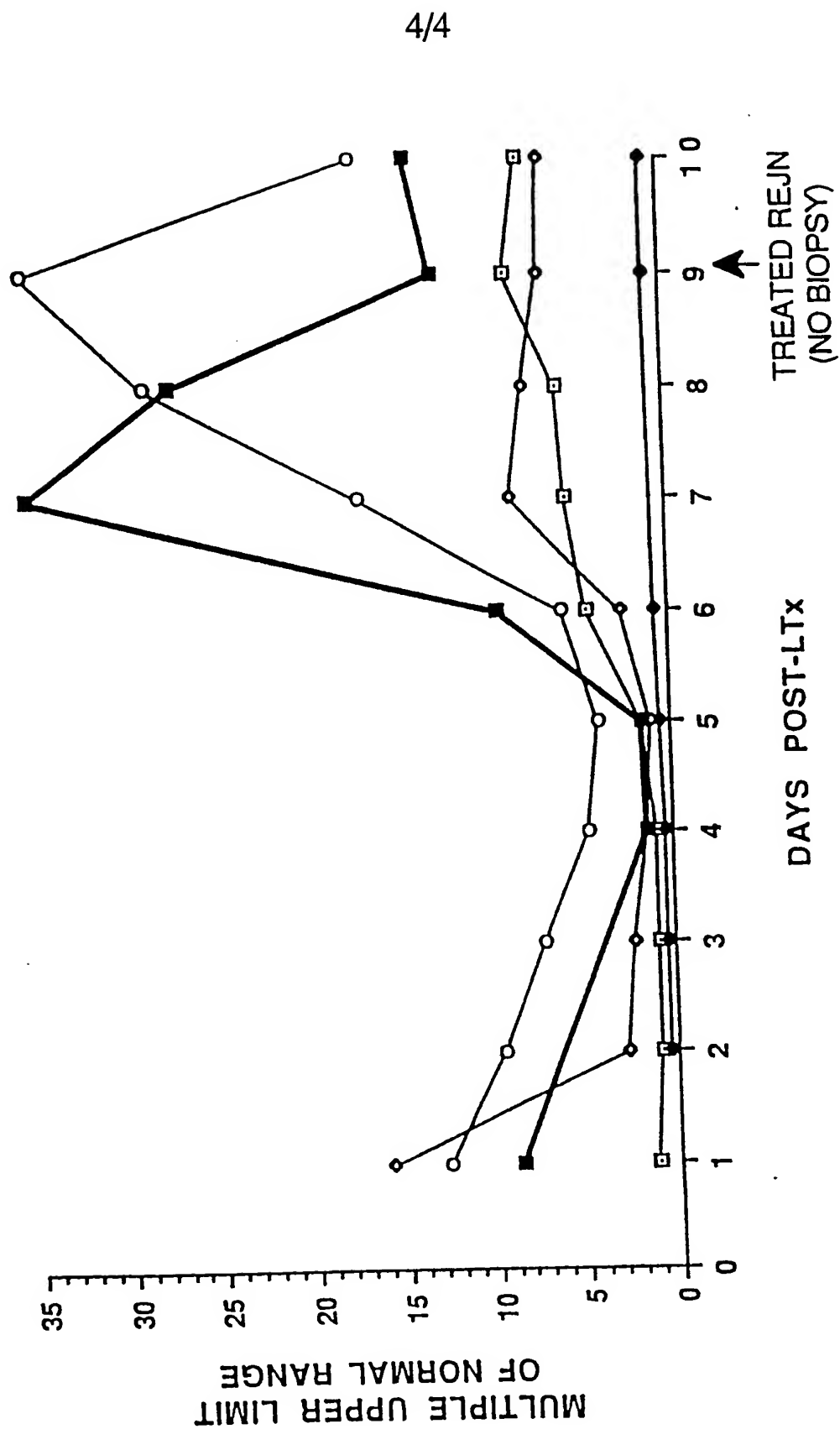


Fig. 4

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/IE 93/00018

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup> According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl. 5 C12Q1/48;                      G01N33/573;                      C07K15/00;                      //C12P21/08														
<b>II. FIELDS SEARCHED</b> <div style="text-align: center; border: 1px solid black; padding: 2px; margin: 5px 0;">Minimum Documentation Searched?</div> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 30%; padding: 5px;">Classification System</td> <td style="padding: 5px;">Classification Symbols</td> </tr> <tr> <td style="padding: 5px;">Int.Cl. 5</td> <td style="padding: 5px;">C12Q ;                      G01N ;                      C07K</td> </tr> </table> <div style="text-align: center; border: 1px solid black; padding: 2px; margin: 5px 0;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched<sup>8</sup></div>			Classification System	Classification Symbols	Int.Cl. 5	C12Q ;                      G01N ;                      C07K								
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Int.Cl. 5	C12Q ;                      G01N ;                      C07K													
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%; padding: 5px;">Category<sup>9</sup></th> <th style="width: 70%; padding: 5px;">Citation of Document,<sup>11</sup> with indication, where appropriate, of the relevant passages<sup>12</sup></th> <th style="width: 20%; padding: 5px;">Relevant to Claim No.<sup>13</sup></th> </tr> </thead> <tbody> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="padding: 5px;">EP,A,0 245 520 (TEIJIN LIMITED) 19 November 1987 see the whole document ---</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-8</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="padding: 5px;">EP,A,0 328 939 (TEIJIN LIMITED) 23 August 1989 see the whole document ---</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-8</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="padding: 5px;">ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS. vol. 245, no. 2, March 1986, NEW YORK, US. pages 543 - 547 I. Y. WANG ET AL. 'Multiple Ya subunits of glutathione S-transferase detected by monoclonal antibodies.' see the whole document --- <div style="text-align: right; margin-top: 10px;">-/--</div></td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-8</td> </tr> </tbody> </table>			Category <sup>9</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>	A	EP,A,0 245 520 (TEIJIN LIMITED) 19 November 1987 see the whole document ---	1-8	A	EP,A,0 328 939 (TEIJIN LIMITED) 23 August 1989 see the whole document ---	1-8	A	ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS. vol. 245, no. 2, March 1986, NEW YORK, US. pages 543 - 547 I. Y. WANG ET AL. 'Multiple Ya subunits of glutathione S-transferase detected by monoclonal antibodies.' see the whole document --- <div style="text-align: right; margin-top: 10px;">-/--</div>	1-8
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A	EP,A,0 245 520 (TEIJIN LIMITED) 19 November 1987 see the whole document ---	1-8												
A	EP,A,0 328 939 (TEIJIN LIMITED) 23 August 1989 see the whole document ---	1-8												
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>9</sup> Special categories of cited documents: <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div>														
<b>IV. CERTIFICATION</b> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; padding: 5px;">           Date of the Actual Completion of the International Search   <div style="text-align: center;">18 JUNE 1993</div> </td> <td style="width: 50%; padding: 5px;">           Date of Mailing of this International Search Report   <div style="text-align: center;">JUN 25 1993</div> </td> </tr> <tr> <td style="padding: 5px;">           International Searching Authority   <div style="text-align: center;">EUROPEAN PATENT OFFICE</div> </td> <td style="padding: 5px;">           Signature of Authorized Officer   <div style="text-align: center;">GRIFFITH G.</div> </td> </tr> </table>			Date of the Actual Completion of the International Search  <div style="text-align: center;">18 JUNE 1993</div>	Date of Mailing of this International Search Report  <div style="text-align: center;">JUN 25 1993</div>	International Searching Authority  <div style="text-align: center;">EUROPEAN PATENT OFFICE</div>	Signature of Authorized Officer  <div style="text-align: center;">GRIFFITH G.</div>								
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International Searching Authority  <div style="text-align: center;">EUROPEAN PATENT OFFICE</div>	Signature of Authorized Officer  <div style="text-align: center;">GRIFFITH G.</div>													

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		Relevant to Claim No.
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	
A	CHEMICAL ABSTRACTS, vol. 105, no. 21, 24 November 1986, Columbus, Ohio, US; abstract no. 189049, K. MATSUMOTO ET AL. 'A monoclonal antibody specific for the Yc subunit of rat liver glutathione S-transferase B.' page 557 ;column 1 ; see abstract & CANCER RES. vol. 46, no. 10, 1986, pages 5259 - 5263	1-8
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**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

IE 9300018  
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This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.  
The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

18/06/93

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